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<b>(51) International Patent Classification <sup>6</sup>:</b> <b>C07K 7/00, 14/435, 16/00, A61K 38/00, 39/00, C12N 5/16</b>	<b>A1</b>	<b>(11) International Publication Number:</b> <b>WO 95/25739</b>
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<b>(21) International Application Number:</b> PCT/US95/03408 <b>(22) International Filing Date:</b> 21 March 1995 (21.03.95)  <b>(30) Priority Data:</b> 08/217,187      24 March 1994 (24.03.94)      US  <b>(71) Applicant:</b> LUDWIG INSTITUTE FOR CANCER RE- SEARCH [CH/US]; 1345 Avenue of the Americas, New York, NY 10105 (US).  <b>(72) Inventors:</b> VAN DER BRUGGEN, Pierre; UCL 7459, Avenue Hippocrate 74, B-1200 Brussels (BE). BOON-FALLEUR, Thierry; UCL 7459, Avenue Hippocrate 74, B-1200 Brussels (BE). TRAVERSARI, Catia; Via Olgettina, 60, I-20132 Milano (IT). FLEISCHAUER, Katharina; Piazzale Lugano, 9, I-20158 Milan (IT).  <b>(74) Agent:</b> HANSON, Norman, D.; Felfe & Lynch, 805 Third Avenue, New York, NY 10022 (US).		<b>(81) Designated States:</b> AU, CA, CN, FI, JP, NO, NZ, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).  <b>Published</b> <i>With international search report.</i>
<b>(54) Title:</b> ISOLATED, MAGE-3 DERIVED PEPTIDES WHICH COMPLEX WITH HLA-A2 MOLECULES AND USES THEREOF		
<b>(57) Abstract</b> <p>Tumor rejection antigens derived from tumor rejection precursor MAGE-3 have been identified. These "TRAS" bind to the MHC-class I molecule HLA-A2, and the resulting complexes stimulate the production of cytolytic T cell clones which lyse the presenting cells. The peptides and complexes may be used diagnostically, therapeutically, and as immunogens for the production of antibodies, or as targets for the generation of cytolytic T cell clones.</p>		

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5           ISOLATED, MAGE-3 DERIVED PEPTIDES WHICH COMPLEX  
            WITH HLA-A2 MOLECULES AND USES THEREOF

FIELD OF THE INVENTION

10           This invention relates to immunogenetics and to peptide  
chemistry. More particularly, it relates to peptides, such as  
nonamers, decamers, and undecamers useful in various ways,  
including immunogens and as ligands for the HLA-A2 molecule.  
More particularly, it relates to a so-called "tumor rejection  
antigen", derived from the tumor rejection antigen precursor  
encoded by gene MAGE-3, and presented by MHC-class I molecule  
15       HLA-A2.

BACKGROUND AND PRIOR ART

20           The study of the recognition or lack of recognition of  
cancer cells by a host organism has proceeded in many  
different directions. Understanding of the field presumes  
some understanding of both basic immunology and oncology.

25           Early research on mouse tumors revealed that these  
displayed molecules which led to rejection of tumor cells when  
transplanted into syngeneic animals. These molecules are  
"recognized" by T-cells in the recipient animal, and provoke  
a cytolytic T-cell response with lysis of the transplanted  
cells. This evidence was first obtained with tumors induced  
in vitro by chemical carcinogens, such as methylcholanthrene.  
The antigens expressed by the tumors and which elicited the T-  
cell response were found to be different for each tumor. See  
30       Prehn, et al., J. Natl. Canc. Inst. 18: 769-778 (1957); Klein  
et al., Cancer Res. 20: 1561-1572 (1960); Gross, Cancer Res.  
3: 326-333 (1943), Basombrio, Cancer Res. 30: 2458-2462 (1970)  
for general teachings on inducing tumors with chemical  
carcinogens and differences in cell surface antigens. This  
class of antigens has come to be known as "tumor specific  
transplantation antigens" or "TSTAs". Following the  
observation of the presentation of such antigens when induced  
by chemical carcinogens, similar results were obtained when  
tumors were induced in vitro via ultraviolet radiation. See  
40       Kripke, J. Natl. Canc. Inst. 53: 333-1336 (1974).

          While T-cell mediated immune responses were observed for

5 the types of tumor described supra, spontaneous tumors were thought to be generally non-immunogenic. These were therefore believed not to present antigens which provoked a response to the tumor in the tumor carrying subject. See Hewitt, et al., Brit. J. Cancer 33: 241-259 (1976).

10 The family of tum<sup>-</sup> antigen presenting cell lines are immunogenic variants obtained by mutagenesis of mouse tumor cells or cell lines, as described by Boon et al., J. Exp. Med. 152: 1184-1193 (1980), the disclosure of which is incorporated by reference. To elaborate, tum<sup>-</sup> antigens are obtained by  
15 mutating tumor cells which do not generate an immune response in syngeneic mice and will form tumors (i.e., "tum<sup>+</sup>" cells). When these tum<sup>+</sup> cells are mutagenized, they are rejected by syngeneic mice, and fail to form tumors (thus "tum<sup>-</sup>"). See Boon et al., Proc. Natl. Acad. Sci. USA 74: 272 (1977), the  
20 disclosure of which is incorporated by reference. Many tumor types have been shown to exhibit this phenomenon. See, e.g., Frost et al., Cancer Res. 43: 125 (1983).

It appears that tum<sup>-</sup> variants fail to form progressive tumors because they initiate an immune rejection process. The  
25 evidence in favor of this hypothesis includes the ability of "tum<sup>-</sup>" variants of tumors, i.e., those which do not normally form tumors, to do so in mice with immune systems suppressed by sublethal irradiation, Van Pel et al., Proc. Natl. Acad. Sci. USA 76: 5282-5285 (1979); and the observation that  
30 intraperitoneally injected tum<sup>-</sup> cells of mastocytoma P815 multiply exponentially for 12-15 days, and then are eliminated in only a few days in the midst of an influx of lymphocytes and macrophages (Uyttenhove et al., J. Exp. Med. 152: 1175-1183 (1980)). Further evidence includes the observation that  
35 mice acquire an immune memory which permits them to resist subsequent challenge to the same tum<sup>-</sup> variant, even when immunosuppressive amounts of radiation are administered with the following challenge of cells (Boon et al., Proc. Natl. Acad. Sci. USA 74: 272-275 (1977); Van Pel et al., supra;  
40 Uyttenhove et al., supra).

Later research found that when spontaneous tumors were

5 subjected to mutagenesis, immunogenic variants were produced  
which did generate a response. Indeed, these variants were  
able to elicit an immune protective response against the  
original tumor. See Van Pel et al., J. Exp. Med. 157: 1992-  
2001 (1983). Thus, it has been shown that it is possible to  
10 elicit presentation of a so-called "tumor rejection antigen"  
in a tumor which is a target for a syngeneic rejection  
response. Similar results have been obtained when foreign  
genes have been transfected into spontaneous tumors. See  
Fearson et al., Cancer Res. 48: 2975-1980 (1988) in this  
15 regard.

A class of antigens has been recognized which are  
presented on the surface of tumor cells and are recognized by  
cytolytic T cells, leading to lysis. This class of antigens  
will be referred to as "tumor rejection antigens" or "TRAs"  
20 hereafter. TRAs may or may not elicit antibody responses.  
The extent to which these antigens have been studied, has been  
via cytolytic T cell characterization studies, in vitro i.e.,  
the study of the identification of the antigen by a particular  
cytolytic T cell ("CTL" hereafter) subset. The subset  
25 proliferates upon recognition of the presented tumor rejection  
antigen, and the cells presenting the antigen are lysed.  
Characterization studies have identified CTL clones which  
specifically lyse cells expressing the antigens. Examples of  
this work may be found in Levy et al., Adv. Cancer Res. 24: 1-  
30 59 (1977); Boon et al., J. Exp. Med. 152: 1184-1193 (1980);  
Brunner et al., J. Immunol. 124: 1627-1634 (1980); Maryanski  
et al., Eur. J. Immunol. 124: 1627-1634 (1980); Maryanski et  
al., Eur. J. Immunol. 12: 406-412 (1982); Palladino et al.,  
Canc. Res. 47: 5074-5079 (1987). This type of analysis is  
35 required for other types of antigens recognized by CTLs,  
including minor histocompatibility antigens, the male  
specific H-Y antigens, and the class of antigens referred to  
as "tum-" antigens, and discussed herein.

A tumor exemplary of the subject matter described supra  
40 is known as P815. See DePlaen et al., Proc. Natl. Acad. Sci.  
USA 85: 2274-2278 (1988); Szikora et al., EMBO J 9: 1041-1050

5 (1990), and Sibille et al., J. Exp. Med. 172: 35-45 (1990),  
the disclosures of which are incorporated by reference. The  
P815 tumor is a mastocytoma, induced in a DBA/2 mouse with  
methylcholanthrene and cultured as both an in vitro tumor and  
a cell line. The P815 line has generated many tum<sup>-</sup> variants  
10 following mutagenesis, including variants referred to as P91A  
(DePlaen, supra), 35B (Szikora, supra), and P198 (Sibille,  
supra). In contrast to tumor rejection antigens - and this is  
a key distinction - the tum<sup>-</sup> antigens are only present after  
the tumor cells are mutagenized. Tumor rejection antigens are  
15 present on cells of a given tumor without mutagenesis. Hence,  
with reference to the literature, a cell line can be tum<sup>+</sup>,  
such as the line referred to as "P1", and can be provoked to  
produce tum<sup>-</sup> variants. Since the tum<sup>-</sup> phenotype differs from  
that of the parent cell line, one expects a difference in the  
20 DNA of tum<sup>-</sup> cell lines as compared to their tum<sup>+</sup> parental  
lines, and this difference can be exploited to locate the gene  
of interest in tum<sup>-</sup> cells. As a result, it was found that  
genes of tum<sup>-</sup> variants such as P91A, 35B and P198 differ from  
their normal alleles by point mutations in the coding regions  
25 of the gene. See Szikora and Sibille, supra, and Lurquin et  
al., Cell 58: 293-303 (1989). This has proved not to be the  
case with the TRAs of this invention. These papers also  
demonstrated that peptides derived from the tum<sup>-</sup> antigen are  
presented by the L<sup>d</sup> molecule for recognition by CTLs. P91A is  
30 presented by L<sup>d</sup>, P35 by D<sup>d</sup> and P198 by K<sup>d</sup>.

PCT application PCT/US92/04354, filed on May 22, 1992  
assigned to the same assignee as the subject application,  
teaches a family of human tumor rejection antigen precursor  
coding genes, referred to as the MAGE family. Several of  
35 these genes are also discussed in van der Bruggen et al.,  
Science 254: 1643 (1991). It is now clear that the various  
genes of the MAGE family are expressed in tumor cells, and can  
serve as markers for the diagnosis of such tumors, as well as  
for other purposes discussed therein. See also Traversari et  
40 al., Immunogenetics 35: 145 (1992); van der Bruggen et al.,  
Science 254: 1643 (1991). The mechanism by which a protein is

processed and presented on a cell surface has now been fairly well documented. A cursory review of the development of the field may be found in Barinaga, "Getting Some 'Backbone': How MHC Binds Peptides", Science 257: 880 (1992); also, see Fremont et al., Science 257: 919 (1992); Matsumura et al., Science 257: 927 (1992); Latron et al., Science 257: 964 (1992). These papers generally point to a requirement that the peptide which binds to an MHC/HLA molecule be nine amino acids long (a "nonapeptide"), and to the importance of the first and ninth residues of the nonapeptide.

Studies on the MAGE family of genes have now revealed that a particular nonapeptide is in fact presented on the surface of some tumor cells, and that the presentation of the nonapeptide requires that the presenting molecule be HLA-A1. Complexes of the MAGE-1 tumor rejection antigen (the "TRA" or nonapeptide") leads to lysis of the cell presenting it by cytolytic T cells ("CTLs").

Attention is drawn, e.g., to, concurrently filed application Serial No. \_\_\_\_\_ to Townsend, et al., and Serial No. \_\_\_\_\_ to Melief, et al., both of which present work on other, MAGE-derived peptides.

Research presented in, e.g., U.S. patent application Serial No. 07/938,334 filed August 31, 1992, and in U.S. patent application Serial No. 073,103, filed June 7, 1993, when comparing homologous regions of various MAGE genes to the region of the MAGE-1 gene coding for the relevant nonapeptide, there is a great deal of homology. Indeed, these observations lead to one of the aspects of the invention disclosed and claimed therein, which is a family of nonapeptides all of which have the same N-terminal and C-terminal amino acids. These nonapeptides were described as being useful for various purposes which includes their use as immunogens, either alone or coupled to carrier peptides. Nonapeptides are of sufficient size to constitute an antigenic epitope, and the antibodies generated thereto were described as being useful for identifying the nonapeptide, either as it exists alone, or as part of a larger polypeptide.

5           These references, especially Serial No. 073,103, showed  
a connection between HLA-A1 and MAGE-3; however, only about  
26% of the caucasian population and 17% of the negroid  
population presents HLA-A1 molecules on cell surfaces. Thus,  
it would be useful to have additional information on peptides  
10       presented by other types of MHC molecules, so that appropriate  
portions of the population may benefit from the research  
discussed supra.

          It has now been found that antigen presentation of MAGE-3  
derived peptides is not limited to HLA-A1 molecules. The  
15       invention set forth, in the disclosure which follows,  
identifies peptides which complex with MHC class I molecule  
HLA-A2. The ramifications of this discovery, which include  
therapeutic and diagnostic uses, are among the subjects of the  
invention, set forth in the disclosure which follows.

20       DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

Example 1

          The sequence of the MAGE-3 gene is known, as per Serial  
No. 037,230, e.g., and PCT/US92/04354, e.g., both of which are  
referred to supra, and are incorporated by reference in their  
25       entirety. Similarly, it is known that HLA-A2 cells,  
transfected with a nucleic acid molecule coding for MAGE-3 are  
lysed by cytolytic T cells (see, e.g. Serial No. 037,230, the  
disclosure of which is incorporated by reference in its  
entirety; also, see U.S. patent application Serial No.  
30       073,103, the disclosure of which is incorporated by reference  
in its entirety).

          These findings suggested a review of the amino acid  
sequence coded for by the MAGE-3 gene, together with the  
scoring system developed by Nijman et al., Eur. J. Immunol.  
35       23: 1215 (1993), incorporated by reference in its entirety, to  
identify peptides derived from MAGE-3 which, putatively, bind  
to the HLA-A2 MHC molecule. This reference, in brief,  
describes a system where "anchor", "strong" and "weak" amino  
acids may be found along a peptide. Anchor positions are at  
40       the second and ninth amino acids. There are three possible  
positions where a strong amino acid can be placed, and four



5 where a weak amino acid may be placed. The maximum score possible for a nonamer is  $6^2 \times 4^3 \times 2^4$ , or 36,864. Such peptides were identified, and are the peptides referred to hereafter.

Example 2

10 The peptides identified via the protocol set forth supra were synthesized using a protein synthesizer, and were dissolved in 0.9% NaCl, 5% DMSO (or 5% DMF for the peptide SEQ ID NO: 3), at 0.5 mM. These peptide solutions were stored at -80°C until ready for use.

15 To determine whether or not peptides bound to HLA-A2 molecules, cell line 174 CEM.T2 (hereafter "T2") was used. This cell line is described by Cerundolo et al., Nature 345: 449-452 (1990), and Spies et al., Nature 348: 744-747 (1990), the disclosures of which are incorporated by reference. This is a cell line deficient in the pathway which supplies  
20 peptides to the endoplasmic reticulum, the site of assembly of MHC class I heterodimers. It can assemble MHC class-I molecules, but these are unstable, and, on cell lysis, dissociate into free heavy and light chains during overnight incubation. The heterodimers can, however, be stabilized in  
25 vitro via addition of appropriate peptide ligands, as per Townsend et al., Nature 340: 443-448 (1989); Townsend et al., Cell 62: 285-195 (1990); Cerundolo et al., supra; Schumacher et al., Nature 350: 703-706 (1991); Elliot et al., Nature 351: 402-406 (1991); Elvin et al., Eur. J. Immunol. 21: 72025-2031  
30 (1991). The thus stabilized molecules can be immunoprecipitated with antibodies specific for the MHC class-I molecule.

35 In light of this background, the T2 cells were washed in serum free IMDM medium, and then  $1.0 \times 10^6$  cells were suspended in the 400 ul of the serum free IMDM medium, together with 100 ul synthetic peptide (final concentration: 0.1 mM, 1% DMSO). The mixture was incubated, overnight, at 37°C. Following incubation, the cells were washed and stained, successively,  
40 with HLA-A2 specific monoclonal antibody BB7.2, and FITC labelled, binding fragments of polyclonal goat anti-mouse IgG. Fluorescence ratio was calculated by the following formula:

5                    Mean fluorescence of the experimental sample  
                      -----  
                      Mean fluorescence of the background

10           This yielded the "mean fluorescence ratio" or MFR. In accordance with Nijman et al, supra, an MFR greater than 1.5 indicates binding to HLA-A2.

15           Five peptides were identified which were predicted to bind specifically to the HLA-A2 molecules. These five were tested in the assay described above, and three of them, i.e., SEQ ID NOS: 1, 3 and 4 were found to bind to HLA-A2 molecules. Each had an MFR value greater than the 1.5 value, i.e.

	<u>Peptide</u>	<u>MFR</u>
	M3-44.53     STLVEVTLGEV (SEQ ID NO: 1)	3.5
20	M3-108.116   ALSRKVAEL (SEQ ID NO: 2)	2.17 (and, less than 1.5)
	M3-195.203   IMPKAGLLI (SEQ ID NO: 3)	2.37
	M3-220.228   KIWEELSVL (SEQ ID NO: 4)	2.37
25	M3-277.286   ALVETSYVKV (SEQ ID NO: 5)	1.8 (and, less than 1.5)

The peptides M3108.116 and M3-277.286 had MFRs less than 1.5 in some of the experimental runs, and were not considered further.

### 30           Example 3

                 The results obtained in Example 2 suggested further experiments, and peptide M3-220-228 was used to generate a cytolytic T cell clone, referred to hereafter as CTL 4.2. The CTL clone was obtained using T2 cells, in accordance with Houbiers et al., Eur. J. Immunol. 23: 2072 (1993), previously incorporated by reference in its entirety.

35           Once the CTL clone was isolated, it was used in a chromium release assay in accordance with Boon, et al., J. Exp. Med. 152: 1184 (1980) the disclosure of which is incorporated by reference in its entirety. In addition to T2 cell, line SK23, which is an HLA-A2 presenting line, was tested. The results are presented below:

5 Effector Cell (E): CTL 4.2  
Target (T): HLA-A2 cell plus SEQ ID NO: 4

E/T RATIO		% <sup>51</sup> CR Release			
	T2	T2 + peptide	SK23	SK23 + Peptide	
10	30	0	91	0	35
	7.5	0	88	-1	33
	1.9	-1	84	-1	14
15	0.5	-1	57	-1	2

These data show that target cells, pulsed with SEQ ID NO: 4, are specifically lysed by the cytolytic T cell clone 4.2. No lysis occurs in the absence of the peptide.

20 The foregoing describes the identification of peptides derived from the MAGE-3 tumor rejection antigen precursor which interact with MHC class I molecule HLA-A2. Of particular interest, and a part of the subject matter of the present invention, are the peptides represented by SEQ ID NO: 3 and SEQ ID NO: 4. These peptides are easily synthesized via Merrifield or other peptide synthesis methodologies, and thus isolated peptides of SEQ ID NO: 3 and SEQ ID NO: 4 are a feature of the invention described herein.

25 The peptides, as indicated, complex with HLA-A2 molecules, and these complexes have been immunoprecipitated, thus leading to another feature of the invention, which is isolated complexes of the HLA-A2 molecule and either one of these peptides.

30 Both the peptides and the complexes are useful in various ways. As was shown, the peptides bind to the HLA-A2 molecule, and thus they are useful in assays to determine whether or not HLA-A2 presenting cells are present in a sample. The peptide is contacted to the sample of interest in some determinable form, such as a labelled peptide (radiolabel, chromophoric label, and so forth), or bound to a solid phase, such as a column or an agarose or SEPHAROSE bead, and the

35

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5 binding of cells thereto determined, using standard analytical methods.

Both the peptides and the isolated complexes may be used in the generation of monoclonal antibodies or cytolytic T cell clones specific for the aforementioned complexes. Those skilled in the art are very familiar with the methodologies necessary to accomplish this, and the generation of a cytolytic T cell clone is exemplified supra. As cancer cells present complexes of MAGE-3 derived peptides of SEQ ID NO: 3 or SEQ ID NO: 4 and HLA-A2, these monoclonal antibodies and cytolytic T cells clones serve as reagents which are useful in diagnosing cancer. The chromium release assay discussed supra is exemplary of assays which use CTLs to determine targets of interest, and the art is quite familiar with immunoassays and how to carry these out.

20 Cytolytic T cell clones thus derived are useful in therapeutic milieus such as adoptive transfer. See Greenberg, J. Immunol. 136(5): 1917 (1986); Reddel et al., Science 257: 238 (1992); Lynch et al., Eur. J. Immunol. 21: 1403 (1991); Kast et al., Cell 59: 603 (1989), all of which are incorporated by reference herein. In this methodology, the peptides set forth supra are combined with antigen presenting cells ("APCs"), to form stable complexes. Many such methodologies are known, for example, those disclosed in Leuscher et al., Nature 351: 72-74 (1991); Romero et al., J. Exp. Med. 174: 603-612 (1991); Leuscher et al., J. Immunol. 148: 1003-1011 (1992); Romero et al., J. Immunol. 150: 3825-3831 (1993); Romero et al., J. Exp. Med. 177: 1247-1256 (1993), and Romero et al., U.S. Patent Application Serial No. 133,407, filed October 5, 1993 and incorporated by reference herein. Following this, the presenting cells are contacted to a source of cytolytic T cells to generate cytolytic T cell clones specific for the complex of interest. Preferably, this is done via the use of an autologous T cell clone, found in, for example, a blood sample, taken from the patient to be treated with the CTLs. Once the CTLs are generated, these are reperfused into the subject to be treated in an amount

5       sufficient to ameliorate the cancerous condition, such as by  
lysing cancer cells, inhibiting their proliferation, etc.

Other aspects of the invention will be clear to the skilled artisan and need not be reiterated here.

10       The terms and expressions which have been employed are  
used as terms of description and not of limitation, and there  
is no intention in the use of such terms and expressions of  
excluding any equivalents of the features shown and described  
or portions thereof, it being recognized that various  
modifications are possible within the scope of the invention.

5

## (1) GENERAL INFORMATION:

(i) APPLICANTS: Van der Bruggen, Pierre; Boon-Falleur,  
Thierry; Traversari, Catia; Fleischauer, Katharina

10

(ii) TITLE OF INVENTION: ISOLATED, MAGE-3 DERIVED  
PEPTIDES WHICH COMPLEX WITH HLA-A2 MOLECULES AND USES THEREOF

(iii) NUMBER OF SEQUENCES: 5

(iv) CORRESPONDENCE ADDRESS:

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(C) CITY: New York City

(D) STATE: New York

(E) COUNTRY: USA

(F) ZIP: 10022

(v) COMPUTER READABLE FORM:

20

(A) MEDIUM TYPE: Diskette, 5.25 inch, 360 kb

storage

(B) COMPUTER: IBM PS/2

(C) OPERATING SYSTEM: PC-DOS

(D) SOFTWARE: Wordperfect

25

(vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER: 08/217,187

(B) FILING DATE: 24-MARCH-1994

(C) CLASSIFICATION: 435

(viii) ATTORNEY/AGENT INFORMATION:

30

(A) NAME: Hanson, Norman D.

(B) REGISTRATION NUMBER: 30,946

(C) REFERENCE/DOCKET NUMBER: LUD 5344

(ix) TELECOMMUNICATION INFORMATION:

(A) TELEPHONE: (212) 688-9200

35

(B) TELEFAX: (212) 838-3884

13

- 5 (2) INFORMATION FOR SEQ ID NO: 1:  
(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 10 amino acid  
(B) TYPE: amino acids  
(D) TOPOLOGY linear  
10 (ii) MOLECULE TYPE: protein  
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

Thr Leu Val Glu Val Thr Leu Gly Glu Val

5

10

15

- (2) INFORMATION FOR SEQ ID NO: 2:  
(i) SEQUENCE CHARACTERISTICS:  
20 (A) LENGTH: 9 amino acid  
(B) TYPE: amino acids  
(D) TOPOLOGY linear  
(ii) MOLECULE TYPE: protein  
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

25

Ala Leu Ser Arg Lys Val Ala Glu Leu

5

30

- (2) INFORMATION FOR SEQ ID NO: 3:  
(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 9 amino acid  
(B) TYPE: amino acids  
35 (D) TOPOLOGY linear  
(ii) MOLECULE TYPE: protein  
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

Ile Met Pro Lys Ala Gly Leu Leu Ile

40

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SUBSTITUTE SHEET (RULE 26)

14

5

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 9 amino acid

(B) TYPE: amino acids

10

(D) TOPOLOGY linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

Lys Ile Trp Glu Glu Leu Ser Val Leu

15

5

(2) INFORMATION FOR SEQ ID NO: 5:

20

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 10 amino acid

(B) TYPE: amino acids

(D) TOPOLOGY linear

(ii) MOLECULE TYPE: protein

25

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

Ala Leu Val Glu Thr Ser Tyr Val Lys Val

5

10



5 We claim:

1. Isolated peptide selected from the group consisting of SEQ ID NO: 3 and SEQ ID NO: 4.
2. The isolated peptide of claim 1, designated SEQ ID NO: 3.
- 10 3. The isolated peptide of claim 1, designated SEQ ID NO: 4.
4. Isolated complex of HLA-A2 and the isolated peptide of claim 1.
5. The isolated complex of claim 4, wherein said peptide is designated SEQ ID NO: 3.
- 15 6. The isolated complex of claim 4, wherein said peptide is designated SEQ ID NO: 4.
7. Isolated cytolytic T cell clone specific for a complex of HLA-A2 and a peptide selected from the group consisting of SEQ ID NO: 3 and SEQ ID NO: 4.
- 20 8. The isolated cytolytic T cell clone of claim 7, wherein said peptide is SEQ ID NO: 3.
9. The isolated cytolytic T cell clone of claim 7, wherein said peptide is SEQ ID NO: 4.
10. Monoclonal antibody which specifically binds to a complex of HLA-A2 and a peptide selected from the group consisting of SEQ ID NO: 3 and SEQ ID NO: 4.
- 25 11. The monoclonal antibody of claim 10, wherein said peptide is SEQ ID NO: 3.
12. The monoclonal antibody of claim 10, wherein said peptide is SEQ ID NO: 4.
- 30 13. Method for treating a subject with a cancerous condition characterized by cancer cells which present a complex of HLA-A2 and a peptide molecule selected from SEQ ID NO: 3 and SEQ ID NO: 4 on their surfaces, comprising administering an amount of the isolated cytolytic T cell clone of claim 7 to said subject, sufficient to lyse said cancerous cells.
- 35 14. The method of claim 13, wherein said peptide is SEQ ID NO: 3.
- 40 15. The method of claim 13, wherein said peptide is SEQ ID NO: 4.

- 5        16. The method of claim 13, wherein said cytolytic T cell clone is derived from autologous cytolytic T cells.
17. Method for identifying a subject with a cancerous condition comprising contacting ex corpore a sample taken from said subject with a reagent specific for complexes of HLA-A2 and a peptide selected from the group consisting of SEQ ID NO: 3 and SEQ ID NO: 4, and determining reaction of said reagent with a cell in said sample as a determination of a cancerous condition.
- 10
18. The method of claim 17, wherein said reagent is a cytolytic T cell clone.
- 15
19. The method of claim 17, wherein said reagent is a monoclonal antibody.

## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US95/03408

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : C07K 7/00, 14/435, 16/00; A61K 38/00, 39/00; C12N 5/16.

US CL : 530/300, 350; 424/185.1; 435/240.2

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 530/300, 350; 424/185.1; 435/240.2

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y, P	US, A, 5,342,774 (BOON ET AL.) 30 AUGUST 1994	1-19
Y	Annual Review of Immunology, vol. 12, issued 1994, V. H. Engelhard, "Structure of peptides associated with Class I and Class II MHC molecules", pages 181-207. See entire document.	1-19
Y	Cell, vol. 74, issued 10 September 1993, J. Ruppert et al., "Prominent role of secondary anchor residues in peptide binding to HLA-A2.1 molecules", pages 929-937. See entire document.	1-19

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A" document defining the general state of the art which is not considered to be of particular relevance	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
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"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&" document member of the same patent family
"O" document referring to an oral disclosure, use, exhibition or other means	
"P" document published prior to the international filing date but later than the priority date claimed	

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10 JUL 1995

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